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DA Project 1617K on Promoting Cotton By-Products in Eastern and Southern Africa

Presentation on

Degossypolizationof cottonseed meal

by

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Degossypolization of cottonseed meal

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Cottonseed

- In India, cotton is grown in an area of around 10 million hectares with a production of around 350 lakh bales (2017-18) (CCI, Mumbai)
- Cottonseed forms 2/3rd portion of the seed cotton.

Cotton crop

- The cottonseed production during 2017-18 is 12 million tonnes (AICOSCA, Mumbai)
- Cottonseed otherwise called "Golden goose" since all the parts of cottonseed has been used as food, feed and other valuable products.

cottonseed

- Cottonseed contains 18% oil and 25% protein.
- The cottonseed meal is rich in essential amino-acids and it is an important animal feed.
- The cottonseed oil is recognized as one of the O.K food as edible oil by American Heart Association (AHA).
- The other products obtained from cottonseed are linters and hulls.

Cottonseed products

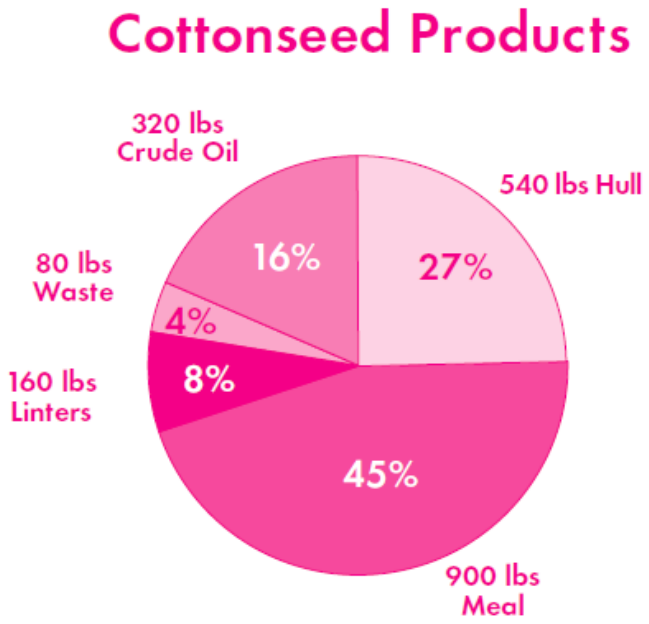


Fig. 1Cottonseed products yield per ton of seed crushed
(National Cottonseed Products Association, 2000)

Table 1. Cottonseed products – Indian Scenario
(All India Cottonseed Crushers Association, 2016)

Cottonseed products	Production (million tonnes)	Uses	Value (million US Dollars)
Whole cottonseed	12	Direct feeding to animals, seed crushing	3380
oil	1.35	Cooking, salad dressings	1045
Linters	0.025	Cellulose, MCC, Cellulose derivatives	10.5
Meal	0.5	Animal feed, High Protein source	141
Hulls	0.15	Roughage, Animal feed	21
Undecorticated cake	9.0	Animal feed	2535

Fig. Comparison of schematic diagram of conventional and scientific processing of cottonseed

oil

Un-decorticated cake

a. Conventional method

Cottonseed

↓

Oil extraction (screw expeller)

↓

Un-decorticated cottonseed cake | Oil

b. Scientific method

Cleaning of the Seed & Delinting (separation of linters)

↓

Dehulling (separation of hulls and kernel)

↓

Drying, Flaking and rolling of the seed kernel and its cooking

↓

Extraction of Oil (screw expeller/ solvent extraction)

↓

De-oiled cottonseed cake | Oil

cottonseed

linters

hulls

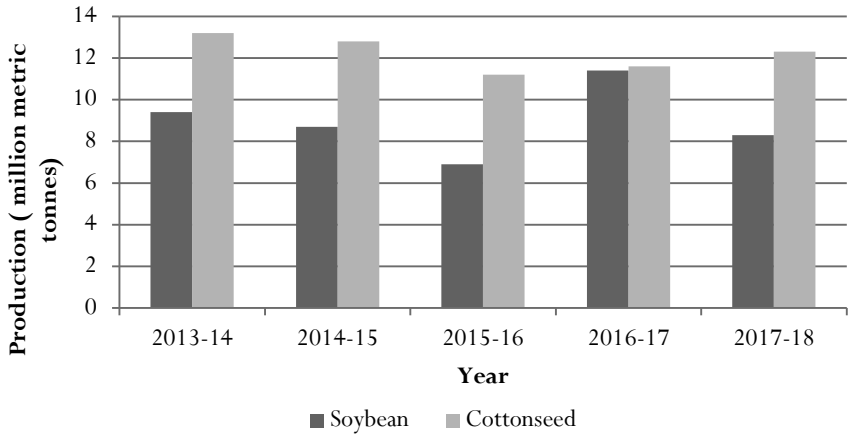
oil

meal

In India, 90 % of cottonseed is processed by conventional method

A comparison of production and price of soybean and cottonseed

- Soybean meal is commonly used as protein source in non-ruminants feed (poultry, fishes, piggeries etc.)
- Considering the stability in production and price, cottonseed meal could be a best alternative substitute to soybean for non-ruminants protein feed source.



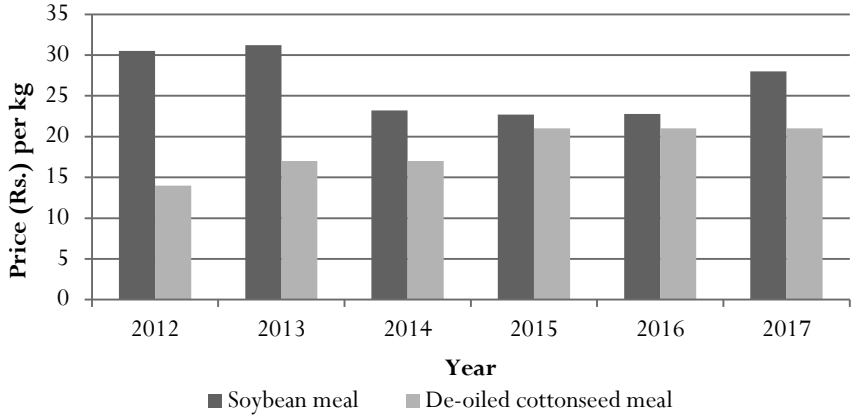


Table. Comparison of nutritive quality of cottonseed meal and soybean meal

S. No.	Parameters (%)	Soybean meal	Cottonseed meal
1.	Crude protein	44-48	30-38
2.	Crude fibre	5-7	12-16
3.	Lysine	2.5 -3.5	0.8-1.2
4.	Gossypol	Nil	Free: 0.08-0.12 Bound: 1.0 to 1.5

Limitations of cottonseed meal in non-ruminants feed

- Presence of toxic compound, gossypol
- Low level of essential amino-acid, lysine
- High fibre content

Gossypol

- Gossypol is a polyphenolic binaphthyl dialdelhyde, and yellow pigment present in entire cotton plant including its seed

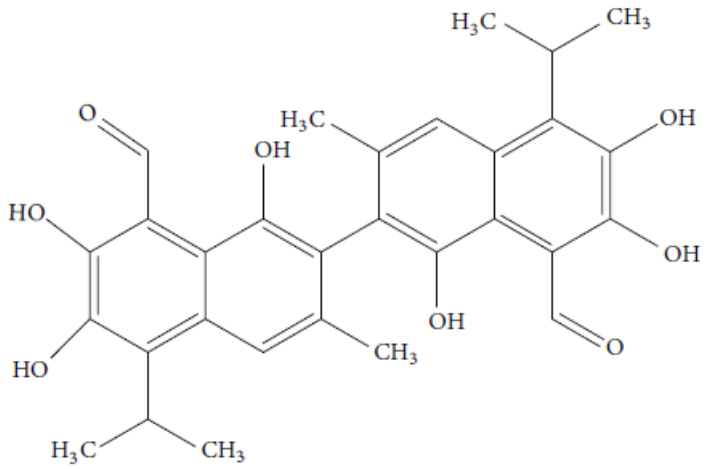
- In cottonseed, Gossypol presents in two forms viz., Free Gossypol (0.4-1.5%) and bound gossypol (2.0-4.0%) (Pons and Eaves, 1967)

- Feeding, diets containing gossypol to animals would cause negative effects such as growth depression, reproductive disease and intestinal and other internal organ abnormalities (Berardi and Goldblatt, 1980; Francis et al., 2001; Robinson et al., 2001).

- The Lethal Dose 50 (LD 50) (mg/kg) value of gossypol in rats, mice, rabbits and pigs are 2400-3340, 500-950, 350-600 and 550 respectively (European Food Safety Authority (EFSA) 2008).

- According to Food and Drug Administration (US FDA) regulations, FG content in feeding diets should be less than 0.045%.

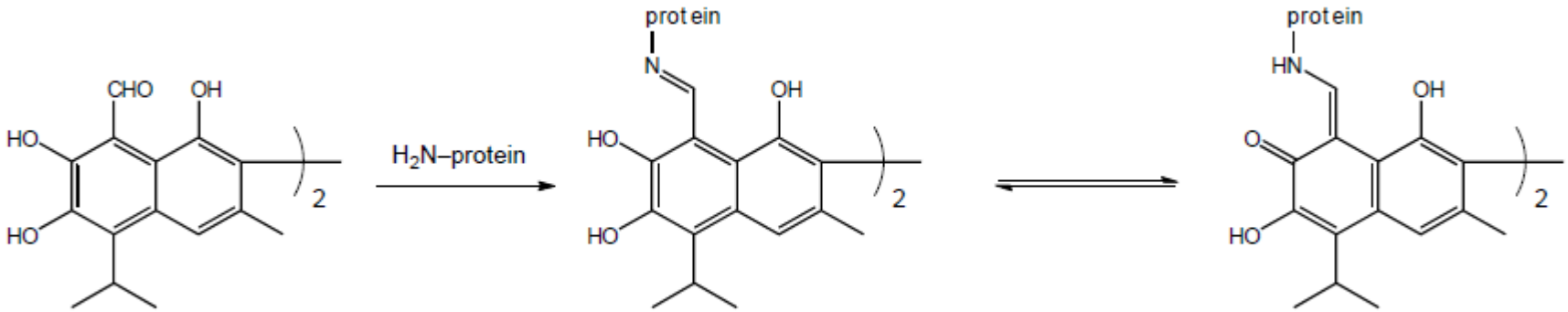
Fig. Structure of Gossypol



(Gadelha eta al., 2014)

Chemical formula: $C_{30}H_{30}O_8$

Chemical structural formula: 2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene)



(EFSA, 2008)

Conjugation of gossypol with amino group of protein forming schiff's base (Bound gossypol)

- **Gossypol binds with epsilon group of amino-acids primarily lysine and possibly arginine and cysteine of proteins during heating in oil extraction and make these amino-acids unavailable to the animals.**

- **Bound gossypol is not toxic however, free gossypol may be released from bound form during digestion in the digestive tract of non-ruminants and may cause toxicity (Gadelha et al., 2014).**

- **A number of chemical methods have been developed for removing gossypol from cottonseed meal such as solvent extraction (Cherry and Gray, 1981; Rahman and Narasingo Rao, 1984), ferrous sulphate treatment (Tabatabai et al., 2002) and calcium hydroxide treatment (Nagalakshmi et al., 2002, 2003). These methods either inactivate FG or converts free to bound form.**

- **Microbial fermentation is a promising method since biodegradation of gossypol occurs during the fermentation process, also the fermented cottonseed meal has reduced bound and free gossypol and enriched with enzymes, vitamins and other active substances (Wu and Chen, 1989; Shi et al., 1998 and Mageshwaran et al., 2016 & 2018).**

Table. A review of methods of degossypolization

Method of degossypolization	Process parameters	Process highlights	Reference	Other remarks
Chemical				
Two stage solvent extraction process	Acetone and Aqueous acetone	Cottonseed protein concentrate (72.2 % protein content)	Gerasimidis et. al., 2007	**Effluent generation,** **High capital investment,** **High capacity processing to be economically viable**
Liquid cyclone process	Liquid classification using hexane	Free gossypol (< 0.045 %), protein content – 65 %	Gastrock et al., 1969	
Acidic solvent extraction	Phosphoric acid in acetone, heated & refluxed	Total gossypol reduction (5 to 10 %),	Pelitire et al., 2014 (https://pubag.nal.usda.gov/download/59559/PDF)	
Solvent extraction	Aqueous acetone extraction	Free and total gossypol reduction	Prons et al., 1967	
Ferrous sulphate treatment	580 mg Fe/kg of diet (40 % cottonseed meal in fish diet)	Free gossypol inactivation	Gaber et al., 2012	
Biological				
Solid state fermentation	*C. tropicalis*	Free gossypol reduction – 95 %, Increase in amino-acid composition	Zhang et al., 2006	**Zero effluent generation,** **Low capital investment,** **Suitable for economically viable small and medium level processing**
	P. flabellatus	70 % Free gossypol and 50 % bound gossypol reduction	Mageshwaran & Kathe, 2013	
	Mixed culture fermentation (*C. tropicalis* + *S. cerevisiae*) and *P. sajor-caju* + *S. cerevisiae*)	80 % Free gossypol reduction 60 % bound gossypol reduction 10 - 20 % crude protein increase 10-15 % crude fibre reduction 10-20% lysine increase	Mageshwaran et al., 2016;2017 & 2018 Shaikh et al., 2014	

Fig. An typical fermentation process (Waites et al., 2001)

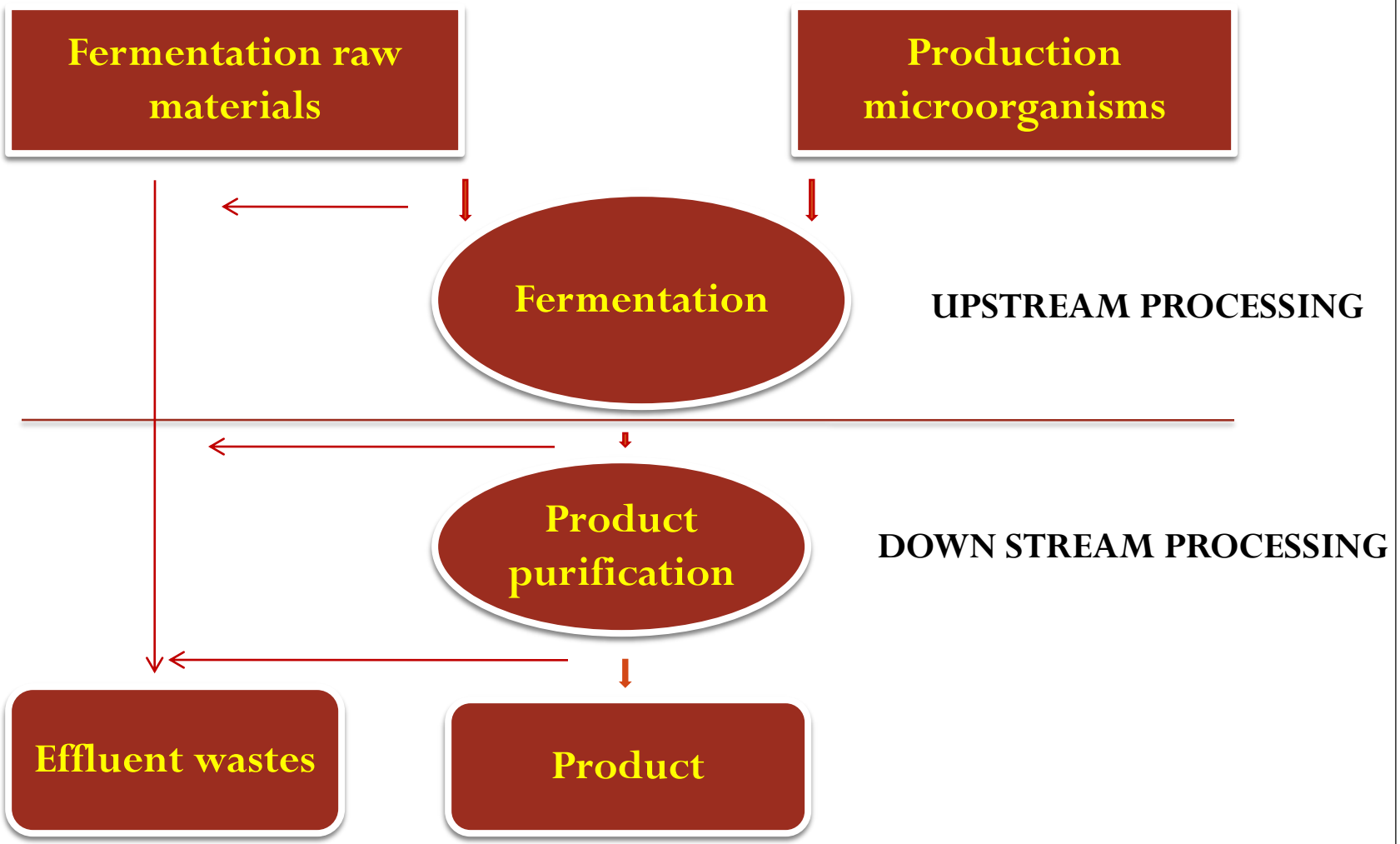


Fig. Interventions of ICAR- CIRCOT 's fermentation process for value-addition to cotton by-products

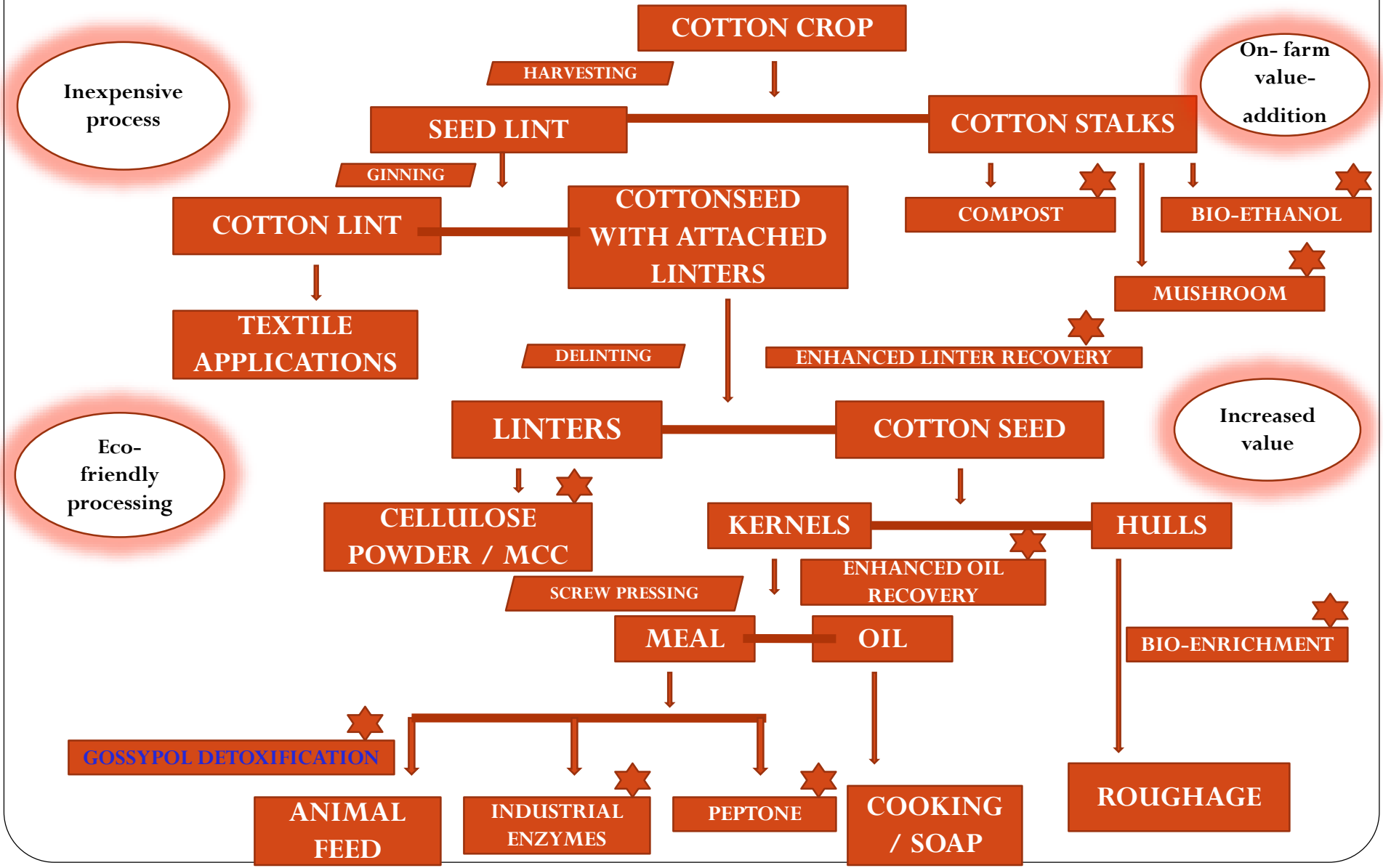
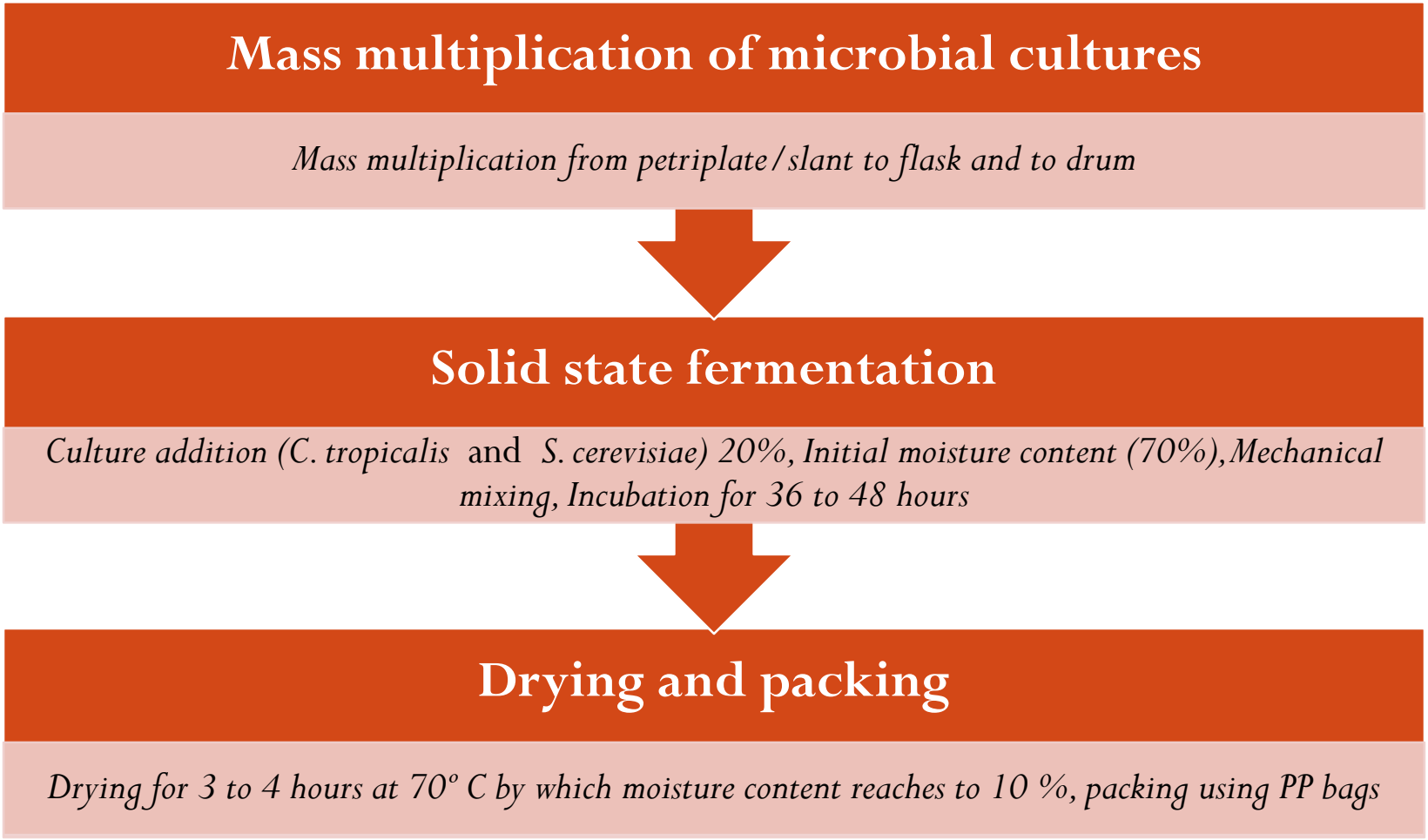


Fig. ICAR-CIRCOT's Degossypolization Process



Steps involved in ICAR- CIRCOT's microbial degossypolization technology

1

1

2

2

3

4

5

Table. Effect of fermentation on degossypolization and nutritive quality improvement of DOC

S. No.	Parameters	Sample	
		Raw DOC	Fermented DOC
1.	Free Gossypol (%)	0.1	0.02
2.	Bound Gossypol(%)	1.2	0.58
3.	Crude Fibre(%)	15	12.0
4.	Crude Protein(%)	32	38.0
5.	Lysine(%)	1.0	1.4

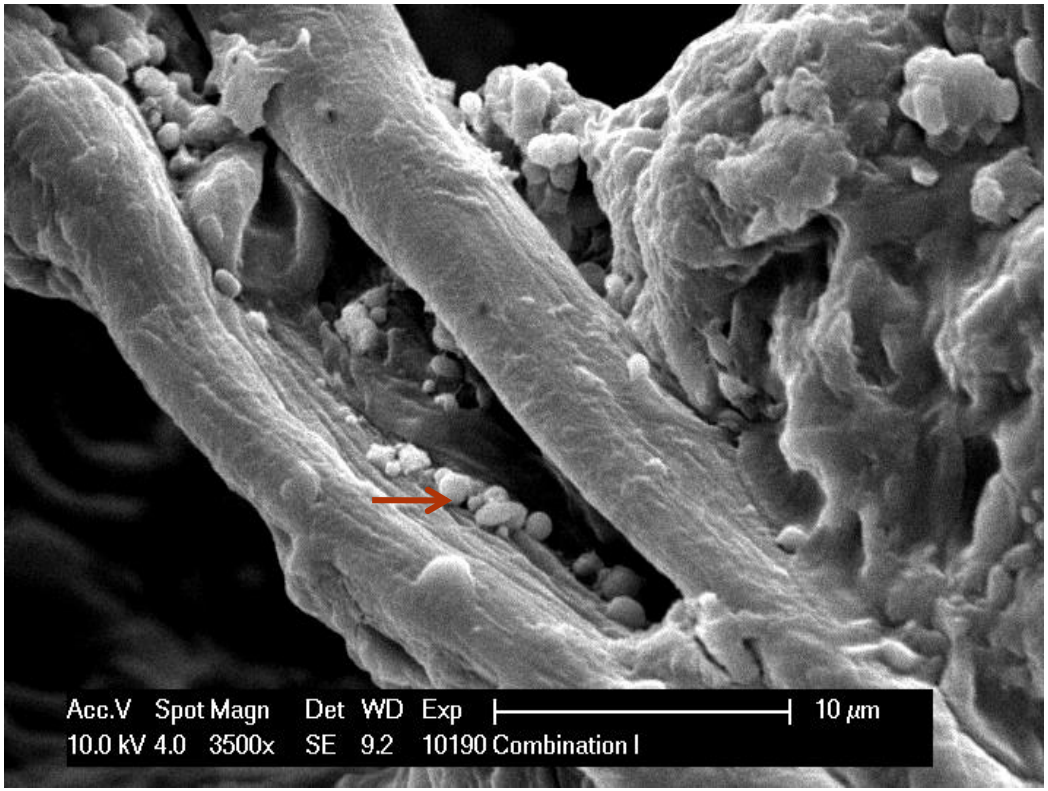
Table. Effect of fermentation on degossypolization and nutritive quality improvement of UD cake

S. No.	Parameters	Sample	
		Raw UD cake	Fermented UD cake
1.	Free Gossypol (%)	0.22	0.045
2.	Bound Gossypol(%)	2.32	0.89
3.	Crude Fibre(%)	37.1	25.6
4.	Crude Protein(%)	20.1	33.5
5.	Lysine(%)	1.0	1.25

Table . Amino acid profile of fermented cottonseed cake

Amino acid (g/100g)	Control	*P.sajor-caju* + *S. cerevisiae* 6933	*C. tropicalis* + *S. cerevisiae*	CD (P= 0.05)
Cystine	4.53^{C}	5.12^{A}	4.70^{B}	0.09
Aspartate	0.76^{B}	0.78^{B}	0.89^{A}	0.09
Threonine	1.99^{B}	2.08^{B}	2.37^{A}	0.14
Serine	0.72^{C}	0.84^{B}	0.98^{A}	0.09
Glutamate	4.49^{A}	3.98^{B}	4.70^{A}	0.23
Glycine	0.72^{B}	0.79^{B}	0.91^{A}	0.09
Alanine	0.83^{B}	0.83^{B}	1.03^{A}	0.14
Valine	0.89	0.84	0.94	NS
Methionine	0.59	0.52	0.57	NS
Leucine + Isoleucine	2.35	2.40	2.37	NS
Tyrosine	1.89^{A}	2.05^{A}	1.27^{B}	0.18
Phenylalanine	**1.04^{B}**	**1.54^{A}**	**1.16^{B}**	**0.15**
Lysine	**0.92^{C}**	**1.36^{B}**	**1.54^{A}**	**0.18**
Histidine	0.46^{B}	0.59^{A}	0.64^{A}	0.09
Arginine	2.41^{B}	2.77^{A}	2.56^{AB}	0.23

Fig. SEM Micrographs showing the presence of yeast cells in fermented cottonseed cake



Note: Arrows indicating the presence of yeast cells

Table. Effect of fermented cottonseed cake on performance in broilers

Parameters	Control	Fermented CSK
Feed Conversion Ratio (FCR)	**1.78**	**1.57**
Mortality* %	**5.00**	**5.00**

- ❖The results showed that culture used in this study are non-pathogenic to broilers
- ❖The FCR was found better in groups received fermented CSK

*Mortality was evaluated by adminsitring culture biomass in drinking water

No. of birds: 20 per treatment; Experimental period: 4 weeks

Table. Economic analysis for the production of degossypolized nutritive enriched DOC

I. Production cost per tonne

S. No.	Input	Cost (US Dollars)
1.	DOC	281.6
2.	Inoculum (Culture)	4.3
3.	Labour cost	12.2
4.	Others (Electricity, water and overhead charges)	5.7
	Total cost	303.8

II. Economics

Parameters	Details
Capital investment	USD 21,126
Fermented cake production/year	250 tonnes per year
Selling price	USD 352 per tonne
Products	Nutritive enriched CSC
Pay-back period	1 year
Running cost	USD 61,972 per annum (cost of raw material, microbial cultures, labour and over heads)

Conclusions :

The following are the salient features of the process

- **70-80 % free gossypol reduction**
- **50-60% bound gossypol reduction**
- **Improved protein content**
- **Enriched lysine content**
- **Eco-friendly process**
- **Zero effluent discharge**
- **A best alternative protein supplement for non-ruminants**

Publications

Intl. J. Food. Ferment. Technol. 6(1): 97-102, June, 2016

DOI: 10.5958/2277-9396.2016.00031.3

RESEARCH PAPER

Optimization of Solid State Fermentation Process for Gossypol Detoxification in Heat Sterilized Cotton Seed Cake by Mixed Fungal Cultures

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Reduction of Gossypol and Increase in Crude Protein Level of Cottonseed Cake using Mixed Culture Fermentation

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Gossypol Detoxification and Lysine Enrichment in Cottonseed Cake by Solid State Fermentation

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Biodegradation of Gossypol by Mixed Fungal Cultures in Minimal Medium[1]

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❖**Received ISCI best research paper award 2014 for the paper entitled " Gossypol detoxification of various cottonseed extractions by fungal cultures during solid state fermentation"**

Technology commercialization

- **Filed a patent (1477/MUM/2014 dated 28-04-14) on " A novel process for gossypol reduction and nutritive quality improvement in cottonseed cake for its use in non-ruminants feed"**

- **The technology licenced to M/s Sana Agro-Industries (Mr. Irfan Ali, Raichur) on non-exclusive basis.**

- **A MoU was signed with M/S Kallam Agro-products pvt. Ltd, Guntur and M/S Bagyalakshmi refinery pvt. Ltd, Tirupur for scale-up trials and commercialization of this technology.**

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THANKYOU.....